Reference: *Biol. Bull.* **180:** 284–294. (April, 1991)

Photoresponsiveness of *Aplysia* Eye is Modulated by the Ocular Circadian Pacemaker and Serotonin

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Abstract. The eye of the sea hare, *Aplysia,* contains a circadian pacemaker that controls rhythmic behaviors of the animal. This report shows that the pacemaker controls the photoresponsiveness of the eye as well. The electroretinogram (ERG) of the isolated eye-optic nerve preparation, evoked by brief green light pulses in otherwise dark conditions, was recorded regularly, while the circadian rhythm of compound action potential activity was continuously recorded from the optic nerve. The waveform of the ERG changed systematically and rhythmically during the circadian cycle. One wave component of the ERG was prominent during the subjective night phase of the rhythm when the compound action potential frequency was minimal; and it was inconspicuous during the subjective day phase of the rhythm when the compound action potential frequency was maximal. Because eyes attached to the central nervous system and isolated eyes both exhibited the same rhythmic ERG changes, the circadian pacemaker in the eye is responsible for modulation of the ERG. Addition of serotonin, a putative efferent transmitter, to the bathing saline induced the ERG wave component characteristic of the subjective night phase of the rhythm. The threshold serotonin concentration was 10^{-7} *M,* and serotonin had a long lasting effect.

Introduction

Each eye of *Aplysia* contains a circadian pacemaker (Jacklet, 1969a) that produces a circadian rhythm in the frequency of optic nerve (ON) autonomous compound action potentials (CAPs). The CAP activity is produced by the synchronous firing of a population of retinal pacemaker neurons (Jacklet *et al.,* 1982), the axons of which enter the optic nerve and project to the central ganglia (Olson and Jacklet, 1985). This rhythm of CAP activity is known to control rhythmic behaviors, such as locomotor activity-rest, because eyeless animals lack the well defined circadian rhythm of activity-rest that normal animals display (Strumwasser *et al.,* 1979; Lickey *et al.,* 1977). Feeding behavior also exhibits a circadian rhythm (Kupfermann, 1974), but the contribution of the ocular circadian pacemaker to that rhythm has not been tested. The ocular circadian pacemaker probably controls rhythmic behavior by neural connections to central motor control centers, or by affecting physiological processes in the eye itself, although the mechanisms are not yet known.

An eye contains the photoreceptors needed to entrain the ocular circadian rhythm to the solar day light-dark cycles, because the rhythm is entrained by light-dark cycles (Eskin, 1971), and the phase of the CAP frequency rhythm is shifted by light pulses given during the subjective night, yielding a phase response curve (Jacklet, 1974). The specific ocular photoreceptor responsible for the phase shifts have not been identified. The eyes and other cephalic photoreceptors also mediate simple phototactic behaviors (see Jacklet, 1980).

The electroretinogram (ERG) has been recorded from the eye by an extracellular pipette placed in the retina (Jacklet, 1969b) and by a suction electrode placed on the cornea (Eskin, 1977; Eskin and Maresh, 1982). The ERG amplitude is increased by serotonin treatment or by ON stimulation, which presumably releases serotonin from the terminal in the eye of the efferent neurons (Eskin and Maresh, 1982). Eyes contain several types of photoreceptors, in addition to the pacemaker neurons involved in the circadian rhythm. The largest and most numerous type is the R photoreceptor (Jacklet and Rolerson, 1982)

Received 9 August 1990; accepted 28 December 1990.

Non-standard abbreviations: CAP, compound action potential; ON, optic nerve; CT, circadian time; ERG, electroretinogram; CM, culture medium.

that responds to light with a graded prolonged depolarization. The light response of this receptor is largely responsible for the ERG (Jacklet, 1969b).

Rhythmic changes in the ERG of *Aplysia*, or any other gastropod, have not been reported, to my knowledge, even though ERG circadian rhythms of other animals are well known. For example, the ERG amplitude rhythm of the compound eye of *Limulus* has been intensively studied (Barlow, 1983). Photosensitivity increases by 20–100 fold during the night, and the rhythm, driven by a central nervous system circadian pacemaker, is mediated by efferent innervation. The efferent transmitter appears to be octopamine (Battelle *et al.*, 1989; Kass and Barlow, 1984).

I report here that the ERG waveform recorded from the isolated *Aplysia* eye changes rhythmically, and the rhythm maintains a stable phase relationship with the circadian rhythm in CAP frequency. Thus, the ocular circadian pacemaker affects physiological processes within the eye itself. The waveform of the ERG that is characteristic of subjective night is induced by the addition of serotonin to the bathing saline during subjective day, mimicking the influence of the circadian pacemaker during subjective night.

Materials and Methods

Individuals of *Aplysia californica* were obtained from Marinus, Inc., Long Beach, California, and kept in Instant Ocean tanks maintained at 16°C under light-dark cycles of 12:12. Two isolated preparations were used: the eye-ON, and the eye attached to the cerebral ganglion by the ON. They were placed in a recording dish (100 ml) equipped with tubing (polyethylene, PE) electrodes embedded in the RTV silicone rubber base. Preparations were maintained in a dark box at 18°C for several days of recording. The ON was drawn, by negative pressure applied by a syringe, into one electrode (PE 10) that was used to record CAPs, and the eye was drawn into another electrode (PE 50) for ERG recording. The negative pressure was released, and the eye and ON remained in place for recording. The eye was drawn completely into the electrode so that the activity of all retinal cells could be recorded.

The eye is spherical and about 0.7 mm in diameter. It has a central lens, a poorly developed cornea, and a complex retina containing photoreceptors and neurons (see Jacklet *et al.*, 1982; Herman and Strumwasser, 1984). ERG electrode recordings routinely picked up small CAPs. Activity was amplified with an A-M Systems model 1700 (gain, ×1000; bandpass 0.1–1000 Hz.) and displayed on a Tektronix 5300 oscilloscope. ERGs and CAPs were recorded and stored with Asystant+ software in an IBM AT computer, and the data were plotted with a Hewlett-Packard model 7470A plotter. The latencies of the ERGs and CAPs were measured from computer generated plots of the recordings.

Pairs of eyes from eight animals were used in the circadian rhythm study. Four eyes attached to the cerebral ganglion yielded 11 circadian cycles of data, and 7 isolated eyes yielded 12 circadian cycles of data. Eyes produced three to four cycles of CAP rhythm data routinely, but complete ERG data were not collected from all eyes. Pairs of eyes from seven animals were used in the serotonin experiments.

Light pulses were produced by driving an Archer green LED with 1–2 s (15, 30 or 70V) pulses from a Grass model S88 stimulator. Pulses were given at regular intervals of 10 min, 30 min, or 1 h in otherwise constant darkness. The LED was positioned 3 cm away and directly over the eye drawn into the PE tubing. The intensity incident on the eye was measured by placing the sensor of a radiometer/photometer (United Detector Corp., Model 40X) 3 cm from the LED. Light intensities for voltage pulses used to drive the LED were 0.6 $\mu W/cm^2$ at 15 V, 0.8 $\mu W/cm^2$ at 30 V, and 3 $\mu W/cm^2$ at 70 V. The *Aplysia* eye has high sensitivity to green light and the threshold intensity is about 0.06 $\mu W/cm^2$ at 500 nm (Jacklet, 1980).

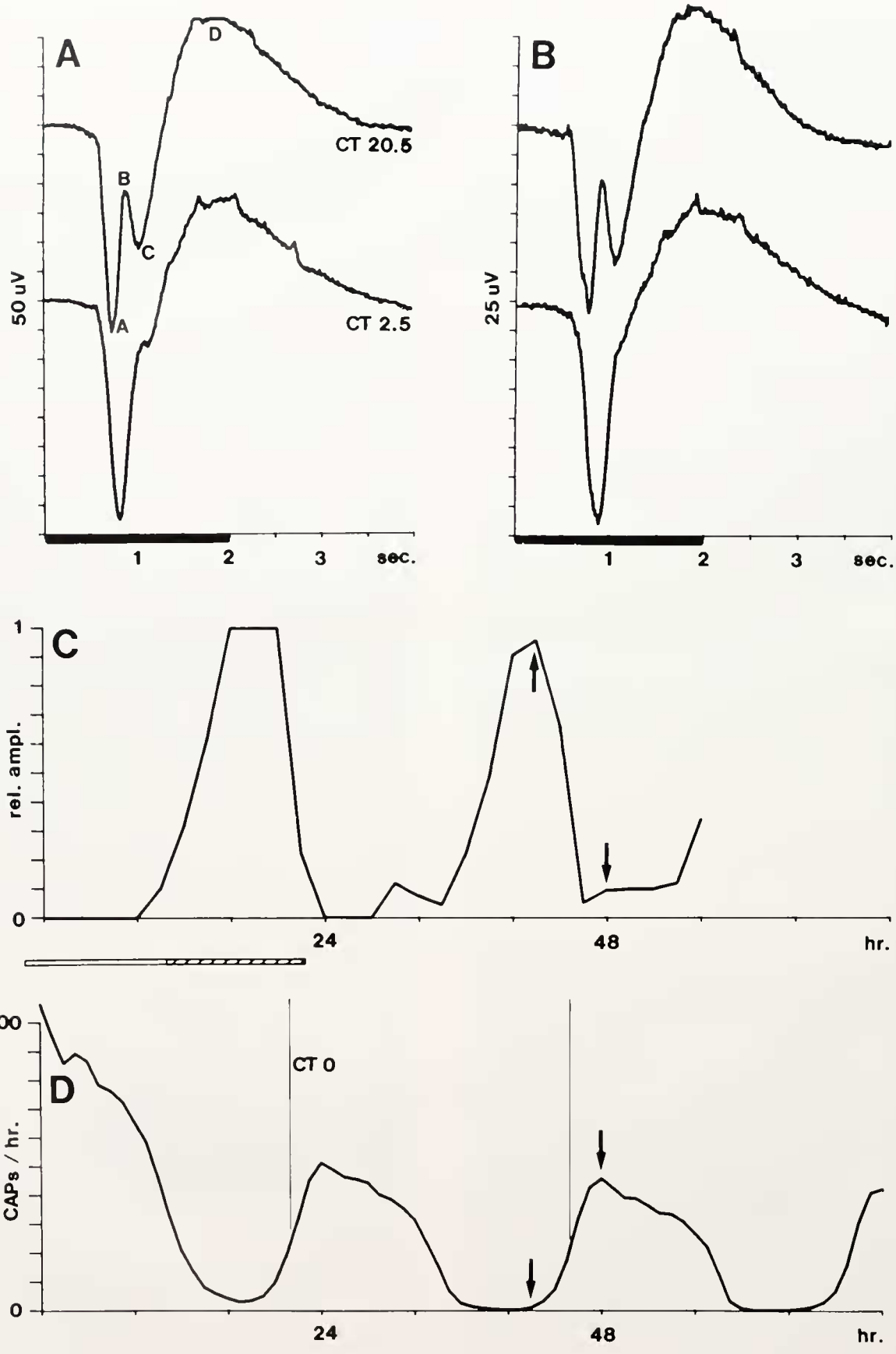
Artificial seawater (ASW) was made up of the following salts in millimoles/liter: NaCl, 425; KCl, 10; $CaCl_2$, 10; $MgCl_2$, 22; $MgSO_4$, 26; $NaHCO_3$, 2.5; adjusted to pH 7.8. Culture medium (CM) was composed of ASW, 20% *Aplysia* blood, and 100 U/ml penicillin, 0.1 mg/ml streptomycin. Serotonin (Sigma, creatinine sulfate) was added to the CM to final concentrations of 10^{-7}, 10^{-6}, or 10^{-5} *M*. A 10-ml chamber fitted with polyethylene tubing for changing solutions inside the dark box was used for the serotonin experiments. The CM was removed entirely by applying suction to the polyethylene tubing and was replaced within a few seconds with the serotonin solution. The statistically significant differences between average latencies were determined using a two tailed t test. The level of significance used was $\alpha = .05$.

Results

Rhythmic changes in the ERG

The basic ERG waveform recorded in these experiments was triphasic, even though the entire eye was pulled into the tubing electrode. The waveform consisted of a sharply rising wave, followed by a slower wave of opposite polarity and a weak slow third phase. There was no obvious "off" response. This triphasic ERG waveform is very similar to the ERG recorded by Eskin (1977) on a Grass polygraph using a suction electrode applied on or near the cornea.

The latency of the ERG in the present study was about 0.9 s in response to a 1 $\mu W/cm^2$ green light pulse. This compares well with the latency of about 0.9 s obtained


A
D
CT 20.5
B
C
CT 2.5
A
50 uV
1
2
3
sec.
B
25 uV
1
2
3
sec.
C
1
rel. ampl.
0
24
48
hr.
500
D
CT 0
CAPs / hr.
0
24
48
hr.

earlier in response to about 6 lux (<1 $\mu W/cm^2$) white light (Jacklet, 1969b). This is a rather long latency, but similar to those of other gastropod eyes under similar conditions. Latency is 1–3 s for *Otala*, a land snail (Gillary and Wolbarsht, 1967), and 0.2–0.5 s for the well-formed eye of *Strombus*, another marine gastropod (Gillary, 1974). The *Aplysia* eye latency is about 0.4 s in response to 600 lux white light (Jacklet, 1969b).

The ERG waveform was usually smooth during the subjective day, as shown in Figure 1A at CT 2.5. CT refers to circadian time, which is measured from the actual period of the free-running circadian rhythm of interest, in this case the CAP frequency rhythm shown in Figure 1D. The period is divided into 24 equal units. Circadian time 0–12 is subjective day, and CT 12–24 is subjective night. The phase point in the CAP rhythm corresponding to subjective dawn (CT 0) is the CAP frequency at ½ maximum marked in Figure 1D. During subjective night, the waveform developed a notch following the initial wave, as shown in Figure 1A at CT 20.5. For convenience, the waveform has been labeled in the figure. The initial wave is A, followed by the B-C wave or notch, followed by the slower D wave. The B-C wave has not previously been reported. This waveform is typical of the ERGs recorded during subjective night in these experiments.

The ERGs shown in Figure 1A were recorded from the same isolated eye at different phases (CT 20.5 and CT 2.5) in the circadian cycle. Similar ERGs were recorded from the other eye of the same animal at the same phases, even though the eye was attached to the cerebral ganglion by the ON (Fig. 1B). Being attached to the cerebral ganglion made no apparent difference in the waveform of the ERG or in the rhythmic changes in the waveform. In general, eye pairs exhibited very similar ERG waveforms, whether or not the eye was attached to the cerebral ganglion.

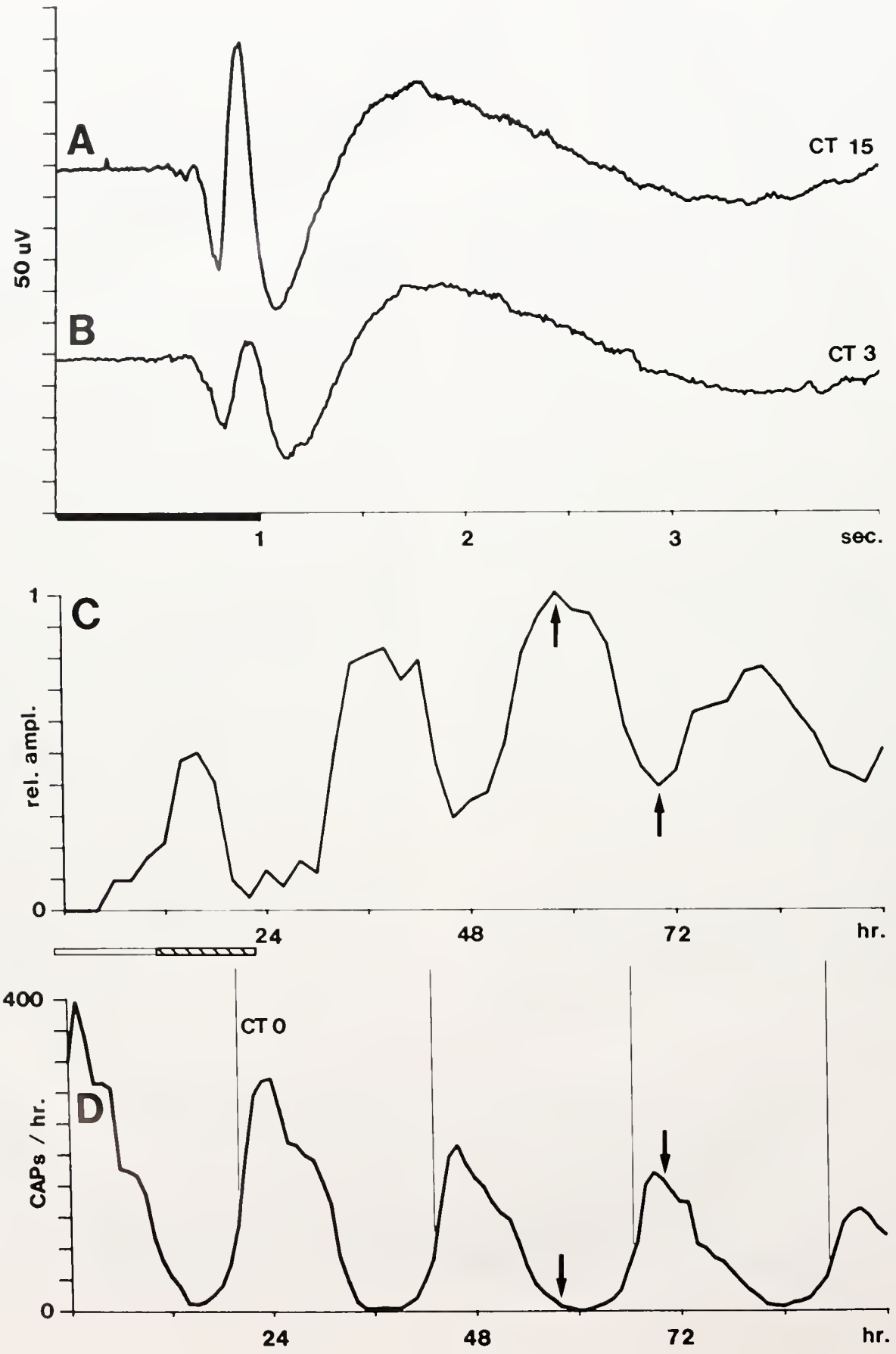
The ERG B-C wave changed rhythmically during the circadian cycle. It virtually disappeared during subjective day and reappeared during subjective night. The relative amplitude of the B-C wave, measured from the peak of the B to the peak of the C wave, cycled continuously, as shown in Figure 1C for the B-C wave of the isolated eye used in Figure 1A. When the cycling of the relative amplitude of the B-C wave is compared to the CAP frequency rhythm plotted in Figure 1D, the maximum B-C wave amplitude seems to occur at about CT 20 and to coincide with minimal CAP frequency during subjective night. The period of both rhythms is about 24 hours.

A few eyes exhibited weak rhythmic changes in the A wave of the ERG, but either they did not persist over two cycles, or they were not sufficiently robust to be considered true rhythms. The D wave was very stable and showed no rhythmicity.

The ERG waveform recorded from eyes of different animals varied somewhat, but the basic waveform could always be observed. One of the most extreme waveforms is shown in Figure 2A, B. This eye was attached to the cerebral ganglion, but the paired isolated eye exhibited the same ERG waveform and changed rhythmically. The A, B, C, and D waves are readily apparent. But the A wave is relatively small and the B-C wave is huge. The relative amplitude of the B-C wave changed rhythmically, but it never completely disappeared. It remained in the appropriate phase relationship with the CAP frequency rhythm for many cycles (Fig. 2C, D). The period of both rhythms is about 23 hours.

In most preparations, the latency (time from stimulus onset to ½ peak of A wave) of the ERG A wave was shorter during the subjective night, when the B-C wave was prominent, than during the subjective day. For example, in Figure 1A and B the latency is about 100 ms shorter during subjective night. The ERG latencies for light pulses given at CT 19–22, during subjective night, were compared to latencies obtained at CT 1–4, during subjective day, for 12 circadian cycles for both isolated eyes and eyes attached to the cerebral ganglion. Mean latency and the standard error of the mean (SEM) were calculated, and *t* tests were performed to determine whether mean differences were statistically significant. The average latency for isolated eyes was 1000 ms (SEM, 20; N, 34) during CT 19–22, and 1050 ms (SEM, 10; N, 39) during CT 1–4. The means were significantly different at the .05 level. The average latency for eyes attached to the cerebral ganglion was 920 ms (SEM, 20; N, 36) during CT 19–22, and

Figure 1. Rhythmic changes in the ERG. ERG waveforms recorded from the same isolated eye during subjective night (CT 20.5) and during subjective day (CT 2.5) are shown in A. The A, B, C, and D waves are labeled. The 2-s light pulse (3 $\mu W/cm^2$) is indicated by the black bar. The other eye from the same animal, but attached to the cerebral ganglion, exhibited the ERG waveform shown in B taken at CT 20.5 and CT 2.5 as in A. Vertical scales in A and B are 50 μV and 25 μV per division. The changes in relative amplitude of the B-C wave of the ERGs recorded from the isolated eye are plotted in C using the same time scale as the CAP frequency rhythm shown in D. Arrows identify the relative amplitude points of the B-C wave in C, and the CAP frequency in D corresponding to the ERGs in panel A taken at CT 20.5 and CT 2.5. Time reference for CT 0 is the thin labeled line in D that occurs at ½ the maximum CAP frequency. The projected light-dark cycle experienced by the animal before dissection is shown by the white/crosshatched bar.


A
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CT 0
D
CAPs / hr.
0
24
48
72
hr.

970 ms (SEM, 20; N, 34) during CT 1–4. The means were significantly different at the .05 level. Although the mean A wave latencies are shorter for attached eyes than for isolated eyes, both show similar shifts in ERG latency during the circadian cycle.

To test for the involvement of chemical synapses in the circadian pacemaker modulation of the ERG waveform, two eyes were subjected to ASW containing 10^{-4} *M* Ca^{++} and 10^{-1} *M* Mg^{++}. This treatment drastically reduced the ERG as expected (Eskin, 1977). Thus, a reliable test for the involvement of chemical synapses was not possible.

Removal of the ON from the isolated eye did not interrupt cycling of the ERG waveform, but it did reduce the number of cycles that an eye exhibited. The ON was cut away from four eyes at the bases of their retinas, and ERGs were recorded as usual. Small CAPs recorded with the ERG electrode verified that the CAP circadian rhythm continued. Two of the eyes remained active for two cycles, and both exhibited cycling of the B-C wave, suggesting that the ON itself is not necessary for circadian cycling of the ERG.

Serotonin induces the ERG B-C wave

Serotonin induced the B-C wave in eyes at circadian times when it was not normally expressed. The induced B-C wave closely resembled the wave characteristic of subjective night. As shown in the example of Figure 3, the B-C wave was well developed at CT 20, as expected, and it became inconspicuous later, at CT 0.5, during subjective day. A short time later at CT 2.0, and just 13 min after the addition of 10^{-6} *M* serotonin to the bathing solution by perfusion, the induced B-C wave (Fig. 3C) was nearly identical to the B-C wave recorded at CT 20 (Fig. 3A). Continued exposure to serotonin enhanced the B-C wave (Fig. 3D) beyond the amplitude of the subjective night B-C wave. The effects of serotonin were long lasting and reached a maximum in about 1 h. Once induced, the B-C wave required several hours of washout before it returned to normal. Serotonin also enhanced the B-C wave of eyes tested during subjective night when the wave was already present.

During exposure to serotonin, the autogenous CAP frequency was reduced as expected from previous work (Corrent *et al.*, 1978; Eskin and Maresh, 1982). The number of CAPs evoked by the light pulse, especially those evoked several seconds after the pulse, were also reduced as shown in Figure 4B. However, compared to the response just before the addition of 10^{-6} *M* serotonin, there was no change in the latency of the initial CAP produced by a light pulse. The mean latency for the initial CAP in the light response was 1.40 s (SEM, 0.14; N, 7) before serotonin treatment, and 1.36 s (SEM, 0.05; N, 7) 15 min after serotonin treatment. A *t* test showed that these means were not significantly different. The initial CAP occurs at about the same time as the B-C wave, and small deflections on the ERG waveform caused by CAPs are clearly visible (Fig. 4A, B). During serotonin treatment, the CAP deflections on the ERG waveform, as well as the size and timing of the initial CAP during the light response, remained the same. Thus, changes in the light-evoked activity of the pacemaker neurons that produce the CAPs are not likely to account for the B-C wave induced by serotonin.

Serotonin shortened the latency of the ERG and occasionally increased the amplitude of the A wave. Both Figures 3 and 4 show a substantial decrease in the latency and increase in the A wave. The average ERG latency before serotonin was 960 ms (SEM, 20; N, 9); 15 min after the addition of 10^{-6} *M* serotonin it was 900 ms (SEM, 30; N, 9), and 45 min after an addition of serotonin it was 860 ms (SEM 40; N, 7). The difference in mean latency was not significant at 15 min, but at 45 min it was significantly different at the .05 level. The average amplitude of the A wave increased only 1.1 times at 45 min. At the threshold concentration (10^{-7} *M*) for ERG B-C wave induction, the average latency decreased by only 25 ms (N, 4), and the A wave amplitude did not change.

Discussion

ERG waveform changes

A major finding of our study is that the ERG changes systematically and rhythmically during the circadian cycle of CAP frequency. One component of the ERG, the B-C wave, is prominent during the subjective night phase of the rhythm when the CAP rate is minimal, and inconspicuous during the subjective day phase when the CAP

Figure 2. Rhythmic changes in the ERG of an eye attached to the cerebral ganglion. Waveforms characteristic of subjective night (CT 15) and subjective day (CT 3) for the same eye attached to the cerebral ganglion by the ON are shown respectively in A and B. The 1-s light pulse (3 $\mu W/cm^2$) is indicated by the black bar. Vertical scales in A and B are 50 μV per division. The B-C wave became very prominent in the eyes of this animal after several days of recording. C shows the changes in relative amplitude of the B-C wave plotted on the time scale of the CAP frequency rhythms in D. Arrows in C and D mark the times at which the ERGs shown in A and B were taken. Time reference for CT 0 is the thin labeled line in D at ½ the maximum CAP frequency. The projected light-dark cycle experienced by the animal before dissection is shown by the white/crosshatched bar. Light pulse is indicated by black bar in panels A, B.

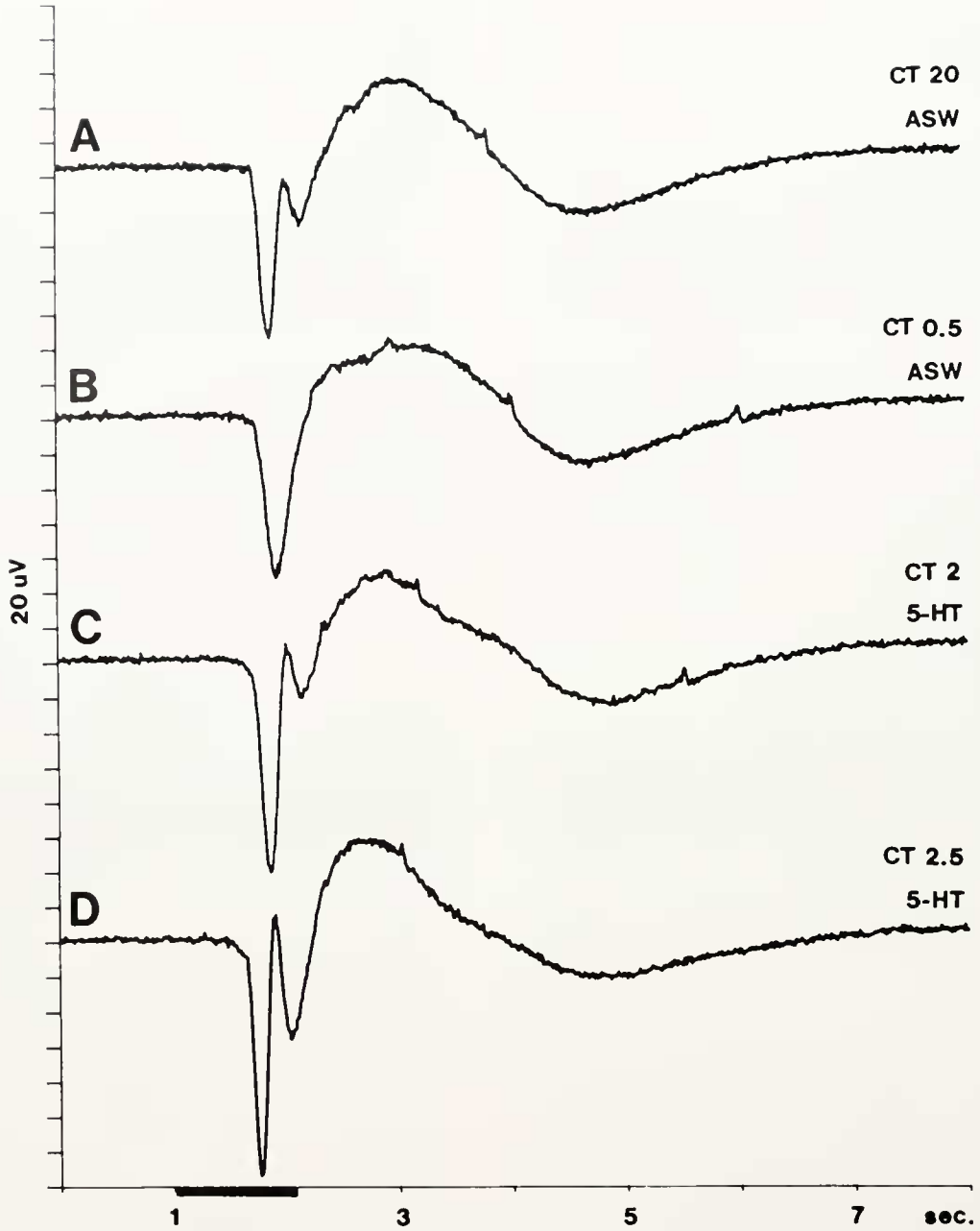

Figure 3. Changes in the ERG of an isolated eye induced by serotonin. The ERG waveform recorded at CT 20 with a characteristic B-C wave is shown in A. At CT 0.5 the ERG had changed to the subjective day waveform, lacking the B-C wave. Thirteen min after the addition of 10^{-6} *M* serotonin, the ERG shown in C with a prominent B-C wave was recorded. The B-C wave progressively increased until the maximum response in D was observed at 43 min after the addition of serotonin. At that time the latency of the A wave had decreased about 100 ms, and the A wave amplitude had increased 140%. Vertical scale is 20 μV per division. The black bar on the time scale marks the 1-s light (.8 μW/cm^2) pulse.

rate is maximal. Thus, the maximal B-C wave occurs during minimal CAP rate in an antiphase relationship. The B-C wave decreases in size sharply during the increase in CAP rate at subjective dawn, suggesting that a high CAP rate might suppress the B-C wave. However, the causal relationship between these events has not been determined. Isolated eyes, and eyes attached to the cerebral ganglion, exhibited similar ERG rhythms suggesting that the B-C wave pacemaker resides within the eye and that it is likely to be the CAP circadian pacemaker. The induction of the B-C wave by serotonin is an intriguing observation that will be addressed later in this discussion.

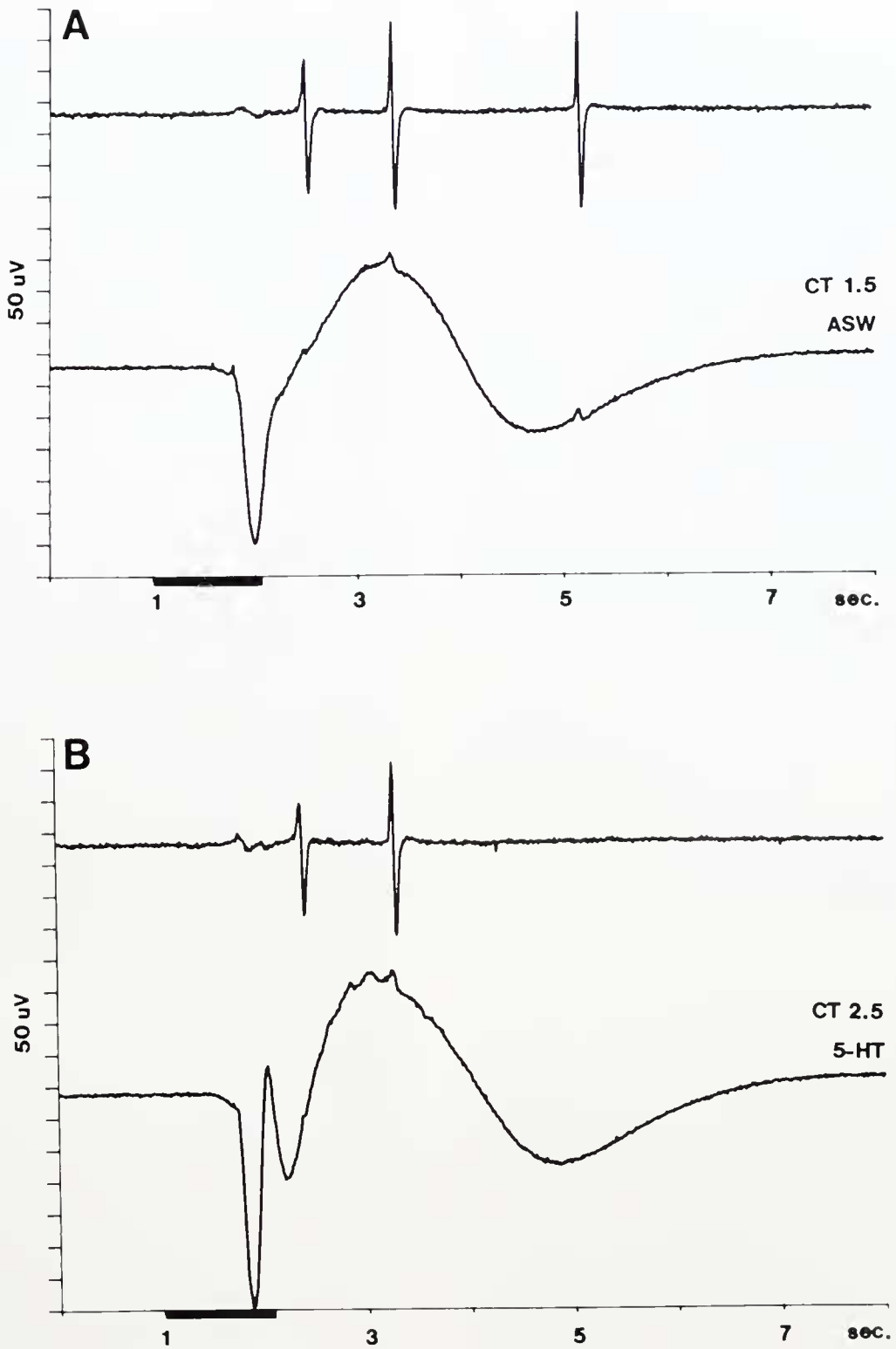

Figure 4. Changes in the ERG and CAP frequency induced by serotonin. Simultaneous recordings were made of CAPs (upper traces) and the ERG (lower trace) in response to 1-s light pulses (dark bar). Responses at CT 1.5 appear in A. CAPs appear on the ON trace and as small deflections on the ERG trace as well. At CT 2.5, 43 min after addition of 10^{-6} *M* serotonin, the B-C wave seen in B was prominent, the latency had decreased about 100 ms, and the number of CAPs evoked decreased. Black bars on time scale mark the 1-s light (.8 $\mu W/cm^2$) pulse. Vertical scales are 50 μV per division.

The ERG B-C wave characteristic of subjective night has not been previously reported. Several years ago, I looked for rhythmic changes in the ERG A wave amplitude because ERG amplitude changes are well known in other animals (see Barlow, 1983), but did not find consistent rhythms. In the present study, the use of computer assisted recording to compare ERG waveforms soon revealed that the B-C wave occurs and changes rhythmically. It also revealed that the A wave latency changes rhythmically, but that the A wave amplitude does not. Average latency differences of 50 ms during the cycle were found for both isolated and attached eyes.

The ERG recordings were made from eyes completely pulled into the tubing electrode, so signals could be recorded from any of the responding cell types in the eye, including cells in the basal retina. Previous work had shown that the largest photoresponses, recorded with a glass pipette in the retina, were corneal negative and were obtained near the distal segments of the large photoreceptors (Jacklet, 1969b). In the present study, light from the LED reached all surfaces of the isolated eye and was not restricted to the pathway through the cornea and lens.

The photoreceptor organization of the *Aplysia* retina was investigated with localized illumination by Block and McMahon (1983). They illuminated (100 lux, white light) the distal segments of the photoreceptors surrounding the lens and, as a result, unitary ON activity without CAPs was evoked in the ON. Illumination of the basal retina produced CAPs. Block and McMahon concluded that chemical synaptic inhibition, especially the inhibitory action of the receptor layer onto the CAP generating neurons, shapes in part the light responses of the isolated eye. Other evidence of synaptic inhibition in the retina is provided below.

Retinal cells that may contribute to the ERG B-C wave

The ERG consists of a sharply rising A wave, a rhythmically changing B-C wave, and a stable D wave. The A wave is likely to be caused by the R type photoreceptors with microvillous distal segments adjacent to the lens (Jacklet, 1969b; Jacklet and Rolerson, 1982). Intracellular recordings show that dark adapted R photoreceptors respond to white light pulses of 600 lux after a latency of 400 ms, comparable to the ERG latency of 400 ms at that intensity (Jacklet, 1969b); the response is a prolonged depolarization of 60–70 mV (Jacklet and Rolerson, 1982). Light adapted, but not dark adapted, R photoreceptors have a notch on the rising phase of the depolarization. The notch is probably not responsible for the B-C wave because it occurs early during the A wave, and because ERGs were recorded at interstimulus intervals of up to 1 h when the R photoreceptor are dark adapted and not expected to have a notch. Some R photoreceptors display prolonged hyperpolarization following the initial depolarization (see Fig. 3 in Jacklet and Rolerson, 1982). However this hyperpolarization is also unlikely to cause the B-C wave because it continues much longer than the B-C wave. Responses from R photoreceptors have not been studied throughout the circadian cycle, especially not during the subjective night when the B-C wave occurs, so changes that might account for the B-C wave have not been observed.

Light responses of R photoreceptors are not completely blocked by low Ca^{++} and high Mg^{++} ASW, but the resting potential is decreased, and the light-evoked depolarization is reduced and prolonged (Jacklet and Rolerson, 1982). This should account for the reduction of the ERG in low Ca^{++} and high Mg^{++} ASW previously observed (Eskin, 1977) and confirmed in this study.

The pacemaker neurons (or secondary neurones, Jacklet *et al.*, 1982) responsible for the CAPs also respond to light. They depolarize and fire synchronous action potentials that are correlated 1:1 with the ON CAPs. As shown in this study, CAPs produce small but observable deflections on the ERG waveform at all phases of the circadian cycle, but they are tiny compared to the B-C wave, and none are synchronized with the B-C wave. The light responses of the pacemaker neurons themselves seem unlikely to contribute to the B-C wave. However, the B-C wave is most conspicuous during the phase of the circadian cycle when the autogenous CAP frequency is low or absent. Perhaps low autogenous CAP activity creates the conditions necessary for the B-C wave to occur. The relationship between autogenous CAP activity and expression of the B-C wave has not yet been tested directly.

A retinal cell type that may contribute to the ERG B-C wave is the H photoreceptor (Jacklet and Rolerson, 1982). Its typical light response is a volley of action potentials followed by brisk hyperpolarization, and then depolarization accompanied by action potentials. The hyperpolarization occurs just after the initial photoresponse, at about the time that the B-C wave of the ERG is occurring. The H cell hyperpolarization appears to be synaptically evoked, because electrical stimulation of the ON evokes a similar sharp hyperpolarization (Jacklet and Rolerson, 1982). This cell type may be involved in shaping the light responses observed by Block and McMahon (1983) during selective illumination. The ERG B-C wave might be produced by enhanced inhibitory synaptic interactions within the retina controlled by the circadian pacemaker. Such enhanced interactions might improve the visual performance of the eye.

A determination of the retinal cell types in *Aplysia* that contribute to the rhythmic B-C wave must await a systematic intracellular study of cellular light responses throughout the circadian cycle, preferably with simultaneous ERG recordings.

The eye of a marine gastropod, *Strombus*, may share some of the features of the *Aplysia* eye photoresponses, including the B-C wave. The ERG exhibits two peaks of negativity that are separable under certain conditions of light and temperature (Gillary, 1974). The second peak resembles the B-C wave. This eye is 3 times the diameter, and contains about 100 times as many cells, as the *Aplysia* eye. It appears to lack circadian pacemaker neurons, and changes in the photoresponse during the circadian cycle have not been explored to my knowledge. This retina contains two types of depolarizing cells and one hyperpolarizing type (Quandt and Gillary, 1979), similar to the *Aplysia* retina. One depolarizing cell type (Type II) exhibits two peaks of depolarization that are similar, but of opposite polarity, to the ERG waveform (Quandt and Gillary, 1980).

Role of serotonin

Serotonin has been shown by Eskin and Maresh (1982) to increase the first wave (A wave in this study) of the *Aplysia* ERG when it is recorded with a suction electrode applied to the cornea. They did not see a B-C wave. That may be due to differences in recording methods, because they made polygraph recordings and did not report latencies. They found an average increase in the ERG amplitude of 63% after 20 min in 10^{-6} *M* serotonin, and a threshold concentration near 10^{-7} *M*. Dopamine, acetylcholine, and octopamine were tested but did not produce consistent ERG changes. They also reported a 20% increase in the ERG in response to ON stimulation and proposed that the stimulation might cause the release of serotonin from efferent terminals in the eye. Terminals have been identified by serotonin antisera (Goldstein *et al.*, 1984; Takahashi *et al.*, 1989).

The effect of serotonin on the ERG suggests that cyclic nucleotide second messengers may be involved. Cyclic AMP mediates many of the serotonin effects on short- and long-term central synapses in *Aplysia* (Kandel and Schwartz, 1982), and serotonin phase shifts the CAP rhythm (Corrent *et al.*, 1978) by a mechanism involving cAMP (Eskin *et al.*, 1982). In addition, cGMP mimics the effect of light on the circadian pacemaker by inducing phase shifts of the CAP rhythm (Eskin *et al.*, 1984). During the cGMP treatment, the membrane potentials of R photoreceptors were not altered, but changes in photoresponsiveness were not explored. A ten minute exposure to light increased the cGMP level by 50%. Cyclic GMP may be elevated, either as a consequence of photoresponses, or because it is involved in the phototransduction process (Eskin *et al.*, 1984). If any rhythmic changes in the levels of cyclic nucleotides occur, they might be involved in the B-C wave and the A wave latency changes.

Serotonin does not appear to induce the B-C wave by a direct effect on the initial light response of pacemaker neurons, because the initial CAP that most nearly coincides temporally with the B-C wave is unaffected by serotonin. Thus, serotonin does not allow expression of the B-C wave by suppressing the light-induced pacemaker neuron activity. However, the phase of the circadian cycle during low or zero CAP frequency is associated with expression of the B-C wave, and serotonin does suppress autogenous CAP activity. Serotonin may create the necessary conditions for expression of the B-C wave, in part, by suppressing autogenous CAP activity.

Because serotonin induces the ERG B-C wave and the reduction of the A wave latency, one may ask how it might be involved in circadian control of the ERG. Serotonin may just be mimicking a natural process, but because there are efferent synaptic terminals containing serotonin in the eye, they may well be involved. Because isolated eyes show circadian rhythms in the B-C wave, the control of serotonin release from the efferent terminals by central neurons is eliminated. But how then might serotonin be released by activity within the eye? Could processes controlled by the circadian pacemaker in the eye release serotonin? Because the appearance of the B-C wave is associated with minimal CAP frequency, release cannot be a direct effect of CAP activity. To produce the appropriate response, autogenous CAP activity would have to suppress serotonin release, and inactivity would have to promote release. Otherwise another process controlled by the circadian pacemaker must be involved.

Acknowledgments

I thank Mark Goldberg for excellent technical assistance. Research supported by NSF grant BNS 88-19773 to J.W.J.

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